Remarks/Arguments

Reconsideration of the above-identified application in view of the present amendment is respectfully requested.

By the present amendment, claims 1 and 57 have been amended to recite that the human $CD133^+/CD34^+$ hemangioblast cells are enriched from umbilical cord blood mononuclear cells. Claim 54 has been amended to correct a typographical error.

Below is a discussion the 35 U.S.C. §103(a) rejection of claims 1-2, 4, 10-12, 21, 23-36, 40-43, 50-54, 56-57 and 62-69.

1. 35 U.S.C. §103(a) rejection of claims 1, 2, 4, 10-12, 21, 23-36, 40-43, 50-54, 56, 57, and 62-69.

Claims 1, 2, 4, 10-12, 21, 23-36, 40-43, 50-54, 56, 57, and 62-69 are rejected under 35 U.S.C. §103(a) as being unpatentable over Strauer *et al.* (2002, *Circulation* 106: 1913-1918) in view of Shake *et al.* (2002, *Annals of Thoracic Surgery* 73: 1919-1926), Ueno *et al.* (U.S. Patent Application Publication No. 2002/0037278), Kawamoto *et al.* (2001, *Circulation* 103: 634-637; reference CJJ on 11/13/06 IDS), Itescu (2003, U.S. Patent Application Publication No. 2003/0199464), and Peichev *et al.* (2000, *Blood* 95: 952-958; reference CZZZ on 4/26/06 IDS).

Applicants respectfully submit that claims 1 and 57 are patentable over Strauer *et al.* in view of Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.* because: (1) the combination of Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.* do not teach or suggest to one of ordinary skill in the art administering to a subject a therapeutically effective

amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells let alone a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells; (2) the Office Action has failed to provide a reasonable rationale to combine Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.* to teach administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells; (3) one of ordinary skill in art would not find it predictable and/or have a reasonable expectation of success in view of Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.* to treat ischemic tissue or induce formation of blood vessels in ischemic tissue by administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells; and (4) the invention recited in claims 1 and 57 exhibits unexpected results.

i. The combination of Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.* do not teach or suggest to one of ordinary skill in the art administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells let alone a a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells.

Strauer *et al.* as discussed in the Office Action teach isolating bone marrow (BM) from human, isolating mononuclear bone marrow cells (BMCs) therefrom, cultivating them overnight, and administering over 10^6 monuclear BMCs to the ischemic tissue using a balloon catheter. Strauer *et al.* also teach that 0.65% of the isolated mononuclear BMCs are AC133+ and that the BMCs include mesenchymal stem cells. Strauer *et al.*, however, do not teach or suggest administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells. BMCs comprising 0.65% endothelial progenitor cells are not a first enriched population of cells comprising 75% human CD133+/CD34+ endothelial progenitor cells and/or a second enriched population of cells consisting essentially of human mesenchymal stem cells.

Shake *et al.* teach isolating MSCs from bone marrow and culturing them such that hematopoietic cells, fibroblasts, and non-MSC adherent cells are washed away, yielding a purified MSC culture. Shake *et al.*, however, do not teach that an enriched population of cells consisting essentially of MSCs can be administered in combination with another enriched population of cells let alone an enriched population of cells comprising 75% human CD133+/CD34+ endothelial progenitor cells.

Ueno *et al.* teach methods of treating ischemic tissues by administering bone marrow mononuclear cells. Ueno *et al.*, however, do not teach or suggest administering to a subject a therapeutically effective amount of a first enriched

population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells. A population of BMCs, which as noted in Strauer *et al.*, comprising 0.65% endothelial progenitor cells, is not a first enriched population of cells comprising 75% human CD133+/CD34+ endothelial progenitor cells and/or a second enriched population of cells consisting essentially of human mesenchymal stem cells.

Kawamoto *et al.*, as discussed in the Office Action, teach administering expanded endothelial progenitor cells (EPCs) to rats in which myocardial ischemia has been induced. The EPCs are selected for CD34+ cells and therefore comprise an enriched population of CD34+ cells. An enriched population of CD34+ however is not a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells.

Itsecu, as discussed in the Office Action, teaches methods of regeneration myocardial tissue after ischemic tissue by administering endothelial progenitor cells that can express CD117, CD34, AC133, or a high level of intracellular GATA activity. Itsecu teaches in the Examples from paragraphs 106+ that a population of CD34+ cells of 98% purity comprising 6-12% CD117^{bright} cells can be administered to a subject. Itsecu, however, does not teach or suggest administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem

cells. Itsecu teaches only that the mononuclear cells can be selected for CD34+ and that these selected and purified cells include at least some cells that include CD133+ markers. Itsecu does not teach selecting for CD133+ and CD34+ to achieve an enriched population of cells that include at least 75% human CD133+/CD34+ endothelial progenitor cells let alone using this population in a therapeutic application in combination with a second enriched population of cells consisting essentially of mesenchymal stem cells.

Peichev *et al.* teach at page 953, first column, last paragraph to second column first paragraph,

> "that a small subset of CD34+ cells derived from hematopoietic sources express AC133 and VEGFR-2…[and] that circulating CD34+ cells expressing VEGFR-2 and AC133 comprise a functional population of CEPs cells that may play a role in postnatal angiogenesis or vasculogenesis."

Peichev *et al.* also note on page 955, column 1, second paragraph, that:

> "CD34^{+} cells isolated from different hematopoietic sources were analyzed by 2-color flow cytometry using PE-conjugated MoAb to AC133 and FITC-conjugated MoAb to VEGFR-2. Almost all of the VEGFR-2^{+} cells derived from mobilized PB that expressed VEGFR-2 also coexpressed AC133 (Figure 2A). However, although mature early-passage HUVECs expressed VEGFR-2, they failed to express AC 133 (Figure 2B, 2C). These data suggest that CD34^{+} cells coexpressing VEGFR-2 and AC133 may represent a phenotypically distinct population of CEPs. The frequency of CD34^{+} cells expressing VEGFR-2 and AC133 in PB was only 0.4 ± 0.2% of the total CD34^{+} population (0.002% of total mononuclear cells). However, with G-CSF mobilization there was an increase in the number of AC133+VEGFR-2+ CEPs to 2.0 ± 0.5% of the CD34^{+} cells (0.02% of total mobilized mononuclear cells). CB- and FL-derived CD34^{+} cells contained 1.4 ± 0.5% and 1.2 ± 0.3% AC133+VEGFR-2^{+} cells, respectively. The number of AC133-CD34^{+}VEGFR-2^{+} cells, which most likely represent mature circulating endothelial cells, composed a very small percentage of the circulating population in mobilized blood. These data demonstrate the existence of low levels but persistent numbers of circulating AC133^{+}VEGFR-2^{+} cells in various hematopoietic sites."

Peichev *et al.* further note on page 956:

> "We have previously shown that surfaces of LVADs in contact with circulating blood are colonized with $CD34^+$ cells with high proliferative capacity, giving rise to a biologically nonthrombogenic neo-intima. Close scrutiny of the LVAD surfaces shows that 4 ± 1% of the mononuclear cells express $AC133^+$ and VEGFR-2^+ cells, suggesting that circulating CEPs have the capacity to colonize neo-intimal surfaces (Figure 6). The presence of $AC133^+$VEGFR-2^+ cells detected on LVADs was more prominent in formed neo-intima explanted early after operation (28 days)."

Accordingly, Peichev *et al.* only teach that it is possible to isolate CD34+CD133+ cells and that CD34+CD133+ cells in circulating blood have the capacity to colonize neo-intimal surfaces. Peichev *et al.* do not teach administering CD34+CD133+ cells to a subject for any purpose let alone to treat ischemic tissue or induce formation of blood vessels nor that an enriched population of CD34+CD133+ cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells can be administered to a subject and would have any potential effect on ischemic tissue or neovascularization alone or in combination with a second population of mesenchymal stem cells. The mere fact the Peichev *et al.* teach CD133+CD34+ cells can be isolated does not teach that such isolated cells can be administered to a subject in a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells.

Accordingly, none of the references cited in the Office Action teach or suggest administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells for any purpose let alone a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells to treat

ischemic tissue or induce formation of blood vessels in ischemic tissue alone or in combination with a second enriched population of cells consisting essentially of human mesenchymal stem cells. Thus, the limitation of administering a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells to treat ischemic tissue or promote neovascularization alone or in combination with a second enriched population of cells consisting essentially of human mesenchymal stem cells is not taught by the combination of references noted in the Office Action.

The Office Action notes that one cannot show nonboviousness by attacking references individually where rejections are based on a combination of references. However, Applicants note that ascertaining the differences between the claimed invention and the prior art is one of the factors in determining whether a given claim is non-obviousness in view of the cited art. *KSR v. Teleflex*, 550 U.S. 398, 406 (2007), citing *Graham v. John Deere Co. of Kansas City*, 383 U. S. 1, 17–18 (1966). Thus, making the above observations as to the failure of the cited references to teach or suggest to one of ordinary skill each and every claim limitation recited in claims, is not attacking the references individually. Instead, the observations made above are meant to point out the shortcomings of the disclosures of Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.* alone, as well as in combination, as it relates the claimed subject matter.

ii. The Office Action has failed to provide a reasonable rationale to combine Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.* to teach administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells.

To overcome the deficiency in the teachings of Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.* as to the administration a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells to treat ischemic tissue or induce formation of blood vessels, the Office Action concludes that:

> "The skilled artisan would have been motivated to enrich the CD34+CD133+EPCs in the administered composition of Strauer *et al.* because Kawamoto *et al.* recognized that EPCs promote neovascularization of ischemic tissue; therefore, administering more cells known at the time of the invention to achieve the desired result of Strauer *et al.* would improve the outcome of the method of Strauer *et al.*"

Applicants fail to see the relevance of this statement as to why one skilled in the art would administer a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells to treat ischemic tissue or induce formation of blood vessels in ischemic tissue. Kawamato *et al.* only teaches administration of an expanded CD34+ endothelial progenitor cells population to treat myocardial infarction. Kawamato *et al.* do not teach that is population is an enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells. As discussed in Applicants' previous response with respect to Kocher *et al.*, less than 24% of mononuclear cells selected for CD34+ cells express AC133. (% of CD34+ cells that are CD117+ x % of CD117^{dim} and CD117^{bright} cells

that are VEGFR-2+). Strauer *et al.*, as discussed above, only teach administering a heterogenous population of BMCs and do not teach administering an enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells or that it is desirable to administer an enriched population of population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells. Peichev *et al.*, as further discussed above, teach only that CD133+CD34+ cells can be isolated and do not teach that such isolated cells can be administered to a subject in an enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells or that is desirable to only administer such isolated cells.

The fact that CD34+ cells can be used to treat a myocardial infarction provides no basis in reason or fact to one skilled in the art that an enriched population of population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells could be used to treat myocardial infarction or "would improve the outcome of the method of Strauer *et al.*" Nor has the Office Action provide an evidence in fact or technical literature to support the conclusory statement that that an enriched population of population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells would improve the outcome of the method of Strauer *et al.*

The Supreme Court of the United States acknowledged that rejections on obviousness grounds cannot be sustained by mere conclusory statements, and that instead, there must be some rational underpinning to support the legal conclusion of obviousness. *KSR Int'l Co. v. Teleflex*, 82 USPQ2d 1385, 1396 (2007). In

particular, to establish a *prima facie* case for obviousness, there must be "a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does." *Takeda Chemical Indus., Ltd. v. Alphapharm Pty. Ltd.,* 492 F.3d 1350, 1356-57 (Fed. Cir. 2007) *quoting KSR Int'l Co. v. Teleflex*, 82 USPQ2d 1385, 1396 (2007). The Office Action has failed to provide a reasonable rationale as to why a skilled would administer an enriched population of population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells to a subject and the obviousness rejection cannot be sustained.

Moreover, it has been held that a claimed composition would have not have been obvious where there was no reason to modify the closest prior art composition to obtain the claimed composition and the prior art taught that modifying the closest prior art would destroy its advantageous properties. *Eisai Co. Ltd. V. Dr. Reddy's Labs., Ltd.*, 533 F.3d 1353 (Fed Cir. 2008).

Here, Strauer *et al.*, which was identified in the Office Action as the closest prior art, teach several different fractions of mononuclear BMCs may contribute to the regeneration of necrotic myocardium and vessels. In order to utilize this large and perhaps heterogeneous regenerative potential, Strauer *et al.* used all mononuclear cells from the bone marrow aspirate as a whole, rather than a subpopulation. Accordingly, there was no reason to modify Strauer *et al.* to use a subpopulation of BMCs especially in view of the other cited prior art because the advantageous properties achieved by using the mixed population of BMCs could be lost. Therefore, it would not be obvious to modify Strauer *et al.* to use a

subpopulation of EPCs let alone an enriched population of population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells.

Additionally, the Office Action fails to provide a reasonable rationale as to why a skilled artisan would administer a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells to treat ischemic tissue or induce formation of blood vessels in combination with a second enriched population of cells consisting essentially of human mesenchymal stem cells.

The Office Action states:

> The person of ordinary skill in the art would have had a further reasonable expectation of success in coadministering the EPCs of Strauer *et al.* with the purified MSCs of Shake *et al.* because the cited references teach that both cells promote healing after myocardial infarction. "It is *prima facie* obvious to combine two compositions each of which is taught by the prior art to be useful for the same purpose, in order to form a third composition to be used for the very same purpose.... [T]he idea of combining them flows logically from their having been individually taught in the prior art." *In* re *Kerkhoven,* 626 F.2d 846, 850, 205 USPQ 1069,1072 (CCPA 1980) (citations omitted). See M.P.E.P. § 2144.06. Since Shake *et al.* teach that their MSCs are "purified" after their culturing step, the level of enrichment would have been a matter of routine optimization at the time of the invention, the skilled artisan recognizing that Shake *et al.* identified a property of MSCs (*i.e.*, cardiac remodeling) and that it would have been desirable to administer as many cells with that property as possible in treating myocardial infarction."

The prior art relied on by Examiner including Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.*, however, neither teaches nor suggests that an enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells is equivalent to (1) a CD34+ EPC population as taught in Kawamato *et al.*, (2) a heterogenous population of

mononuclear BMCs as taught in Strauer *et al.*, or (3) an isolated MSC population as taught in Shake *et al.*

It has been held that an examiner's reliance on equivalents as a rationale supporting an obviousness rejection is inappropriate without evidence that the equivalency was recognized in the prior art. *See In re Ruff,* 256 F.2d 590, 599 (CCPA 1958) ("The equivalence must be disclosed in the prior art").

This analysis is consistent with *KSR,* which notes that:

> [a]lthough common sense directs one to look with care at a patent application that claims as innovation the combination of two known devices according to their established functions, it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed new invention does.

KSR, 550 U.S. at 418. In the instant case, the method of Claim 1 is directed to administering an enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells not CD34+ EPCs or heterogenous mononuclear BMCs. There is no discussion in the prior art cited by the Examiner that an enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells is equivalent to or has even has similar effects as an enriched CD34+ population or a heterogenous mononuclear BMC population in treating ischemic tissue or inducing formation of blood vessels. Moreover, the Examiner has not identified any reason which would have prompted the ordinary artisan to select an enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells for treating ischemic tissue or promoting neovascularization in ischemic tissue. Accordingly, the Office Action has failed to provide a reasonable rationale as to why a skilled would administer to a subject an

enriched population of population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells in combination with a second enriched population of cells consisting essentially of human mesenchymal stem cells.

iii. <u>One of ordinary skill in art would not find it predictable and/or have a reasonable expectation of success of treating ischemic tissue or inducing formation of blood vessels in ischemic tissue by administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells.</u>

In *KSR*, the Supreme Court stated that when an obvious modification "leads to the anticipated success," the invention is likely the product of ordinary skill and is obvious under 35 U*.S.C.* § *103,* 127 S. *Ct. at 1742.* "[O]bviousness cannot be avoided simply by a showing of some degree of unpredictability in the art so long as there was a reasonable probability of success." *Pfizer, 480 F.3d at 1364* (citing *In re Corkill,* 771 *F2d 1496, 1500 (Fed. Cir. 1*985)).

Cases following *KSR,* however, have found that obviousness is not found where the prior art [gives] either no indication of which parameters [are] critical or no direction as to which of many possible choices is likely to be successful" *In re O'Farrell, 853 F.2d* 894, *903 (Fed. Ci*r. 1988). In such cases, "courts should not succumb to hindsight claims of obviousness. "*In re Kubin,* 561 *F.3d 1351, 1359 (Fed Cir. 2009).* Similarly, patents are not barred just because it was obvious "to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it." *In re O'Farrell, 853 F.2d at 903.*

As discussed above, Strauer *et al.* give only general guidance to methods of treating to treating myocardial infarction using mononuclear BMCs and do not specifically teach treating ischemic tissue or inducing formation of blood vessels in a subject by administering an enriched population of population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells in combination with a second enriched population of cells consisting essentially of human mesenchymal stem cells.

Strauer *et al.* discuss at pages 1916-1917 that:

> In recent years, several laboratories have shown that environmentally dictated changes of fate (transdetermination) are not restricted to stem cells but may also involve progenitor cells at different steps of a given differentiation pathway (transdifferentiation). Moreover, mesenchymal stem cells may represent an ideal cell source for treating different diseases. Adult, mononuclear BMCs contain such stem and progenitor cells (≤1%), ego mesodermal progenitor cells, hematopoietic progenitor cells, and endothelial progenitor cells. In several animal infarction models it has been shown that: (1) Bone marrow hemangioblasts contribute to the formation of new vessels; (2) bone marrow hematopoietic stem cells differentiate into cardiomyocytes, endothelium, and smooth muscle cells; (3) BMCs give rise to mesodermal progenitor cells that differentiate to endothelial cells; and (4) endothelial progenitors can transdifferentiate into beating cardlomyocytes. Thus, several different fractions of mononuclear BMCs may contribute to the regeneration of necrotic myocardium and vessels. In order to utilize this large and perhaps heterogeneous regenerative potential, we decided to use all mononuclear cells from the bone marrow aspirate as a whole, rather than a subpopulation. No further expansion was performed because experimental data have revealed a dramatic decline in the homing capacity of in vitro amplified hematopoietic stem or progenitor cells."

Thus, Strauer *et al.* teach that at the time of the filing of the present application it was unknown which fraction of mononuclear BMCs contribute to

regeneration of necrotic myocardium and therefore all mononuclear BMCs were administered instead of enriched fraction of one population.

Itsecu, as discussed above, teaches only that the mononuclear cells can be selected for CD34+ and that these selected and purified cells include at least some cells that include CD133+ markers. Itsecu does not teach selecting for CD133+ and CD34+ to achieve an enriched population of cells that include at least 75% human CD133+/CD34+ endothelial progenitor cells let alone using this population in a therapeutic application in combination with a second enriched population of cells consisting essentially of mesenchymal stem cells.

Peichev *et al.* only teach that it is possible to isolate CD34+CD133+ cells and that CD34+CD133+ in circulating blood have the capacity to colonize neo-intimal surfaces and that CD34+CD133+ can form endothelial cells under the right microenvironment. Peichev *et al.* do not teach administering CD34+CD133+ cells to a subject for any purpose let alone to treat ischemic tissue or induce the formation of blood vessels. The fact that CD34+CD133+ endothelial prgenitor cells can migrate along with other CD34+ cells and potentially form endothelial cells does not show that such endothelial cells will treat the ischemic tissue or promote the formation of blood vessels. Further, Peichev *et al.* provide no teaching to one skilled in the art that an enriched population of CD34+CD133+ cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells can be administered to a subject and would provide any potential effect on ischemic tissue alone or in combination with a second population of mesenchymal stem cells.

Moreover, one of ordinary skill in art have not found it predictable and/or had a reasonable expectation of success that ischemic tissue could be treated by an enriched population of CD34+CD133+ cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells. As discussed above, Strauer *et al.* teach using a mixed population mononuclear BMCs as several different fractions of mononuclear BMCs may contribute to regeneration myocardial tissue. Itesecu teaches selecting for CD34+ cells and administering these cells in combination with a chemokine but does not teach an enriched population of CD34+CD133+ cells comprising at least 75% human CD133+/CD34+ endothelial would have an effect. Peichev *et al.* show only that LVAD surfaces are colonized with CD34+ cell and that of these CD34+ cells, 4% on neo-intimal surfaces are CD133+ cell.

Thus, at the time of filing present application, it was not known that administration of an enriched population of CD34+CD133+ cells comprising at least 75% human CD133+/CD34+ endothelial cells to a subject would have any effect on treating ischemic tissue or inducing the formation of blood vessels in the ischemic tissue. One skilled in the art could not infer, find it predictable, and/or have a reasonable expectation of success that administering to a subject a therapeutically effective amount of a first enriched population of cells comprising at least 75% human CD133+/CD34+ endothelial progenitor cells and a second enriched population of cells consisting essentially of human mesenchymal stem cells would treat the ischemic tissue and/or induce the formation of blood vessels in the ischemic tissue. It has been held that a claim is not obvious where the improvement of the prior art is more than a predictable use of prior art elements according to their known

function or use. *In re Kubin,* 561 F.3d 1351, 1359 (Fed Cir. 2009). Therefore, claims 1 and 54 are not obvious in view of Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.*

iv. <u>The invention recited in claims 1 and 57 exhibits unexpected results.</u>

Assuming arguendo, that claims 1 and 57 were obvious in view of Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.* claims 1 and 57 would still be patentable over Strauer *et al.*, Shake *et al.*, Ueno *et al.*, Kawamoto *et al.*, Itescu *et al.*, and Peichev *et al.* because the subject matter of claims 1 and 57 exhibit unexpected results.

It is well settled that one may rebut a prima facie case of obviousness based on unexpected results by demonstrating that the claimed inventions exhibits some superior property or advantage that a person of ordinary skilled in the art would have found surprising or unexpected. *P&G v. Teva Pharmaceuticals*, 566 F.3d 989, 999 (Fed. Cir. 2009); *citing In re Soni*, 54 F.3d 746, 750 (Fed. Cir. 1995).

Example 11 of the present application describes a series of experiments that were performed to determine whether stromal elements added to UCB-derived EPC would augment neovascularization in the mouse hind limb ischemia model.

> "Augmented neovascularization in the mouse hind-limb injury model by EPC derived from purified UCB CD133+ cells supplemented with human mesenchymal stem cells (hMSC).
>
> A series of experiments was performed to determine whether stromal elements (*e.g.,* hMSC) added to UCB-derived EPC would augment neovascularization in the mouse hind-limb ischemia model.
>
> 1) Isolation and Culture Expansion of hMSCs
>   hMSCs from adult human bone marrow were isolated and expanded in culture as described in Example 6.
> 2) Isolation and Culture Expansion of CD133+ Cells from UCB
> $CD133^+$ from UCB were selected as described in Example 3. After selection, the cells were seeded at

50,000-70,000 cells/well in 96 well plates under the same endothelial-driving culture conditions as described in Example 4. Cell yields ranged from 58-130% of plated CD133+ cells

3) Neovascularization in the Mouse Hind-Limb Injury Model by EPC Derived from Purified UCB CD133+ cells supplemented with hMSCs.

After 7 days of culture, 1×10^6 CD133+ cells and 1×10^6 hMSC were co-injected intracardially into mice that had undergone hind-limb femoral artery ligation by the method described in Example 2. Blood flow was measured by laser Doppler flowmeter over time, and the results illustrated in FIG. 17 are expressed as the ratio between the blood flow in the injured and the uninjured leg over time. The results show increased blood flow in the mouse receiving both CD133+ cells and hMSC cells at day 7 after surgery compared with mice infused with CD133+ cells (day 14) or hMSC alone. This result suggests that improved blood flow was achieved at an earlier time point (day 7) after co-infusion of hMSC with CD133+ cells."

Additionally, attached herewith is redacted draft of grant application presented as Attachment A showing UCB 133+ HSC and human MSC synergistically promote vasculogenesis in response to ischemia.

"Preliminary studies conducted in Dr. Laughlin's laboratory at Case Western Reserve University tested human bone marrow (BM)-derived mesenchymal stem cells (MSC) injected alone and in combination with UCB-derived $CD133^+$ HSC. Significantly enhanced blood flow and histologic evidence of angiogenesis was noted in this NOD.SCID *in vivo* hindlimb femoral ligation model (Fig 2). Femoral ligation and resection was performed and study animals were randomized to one of three treatment groups. Group 1, control, was treated with injection of media (0.02 ml). Group 2 animals received third passage human MSC (1×10^6 in 0.02 ml). Group 3 animals received both UCB $CD133^+$ HSC and MSC at an equivalent total cell dose ($0.5 \times 10^6 + 0.5 \times 10^6$ in 0.02 ml; total combined human cell dose 1×10^6 in 0.04 ml). The animals were survived for 6 weeks. There were significant differences in the Doppler blood flow ratio measured at day 42 among the three conditions. Pair-wise comparison revealed significantly higher blood flow measured in animals injected with both MSC and UCB 133^+ HSC compared with those animals treated with MSC alone ($p<0.05$). Taken together, these preliminary reports point to potential synergy between endogenous endothelial, inflammatory, and stromal cells and administered hematopoietic stem and

> mesenchymal cells in mediating murine angiogenesis responses to ischemic vessel injury."

Thus, Example 11 and the attached redacted grant demonstrate that administering an enriched population of UCB CD133+ cells in combination with an enriched population of human mesenchymal can synergistically promote vasculogenesis in ischemic tissue in comparison to the administration of UCB CD133+ cells alone and MSCs alone. Therefore, the methods recited in claims 1 and 57 exhibit unexpected results and withdrawal of the rejection of claims 1 and 57 is respectfully requested.

Claims 2, 4, 10-12, 21, 23-36, 40-43, 50-53, 56 and 67-69 depend directly or indirectly from claim 1 and are therefore allowable because of the aforementioned deficiencies in the rejection with respect to claim 1 and because of the specific limitations recited in claims 2, 4, 10-12, 21, 23-36, 40-43, 50-53, 56 and 67-69.

Claim 54 includes similar limitations to claim 1 and therefore is allowable because of the aforementioned deficiencies in the rejection with respect to claim 1 and because of the specific limitations recited in claim 57.

Claim 55 depends directly or indirectly from claim 54 and is therefore allowable because of the aforementioned deficiencies in the rejection with respect to claim 54 and because of the specific limitations recited in claim 55.

In view of the foregoing, it is respectfully submitted that the present application is in a condition of allowance and allowance of the present application is respectfully requested.

Please charge any deficiency or credit any overpayment in the fees for this matter to our Deposit Account No. 20-0090.

Respectfully submitted,

/Richard A. Sutkus/
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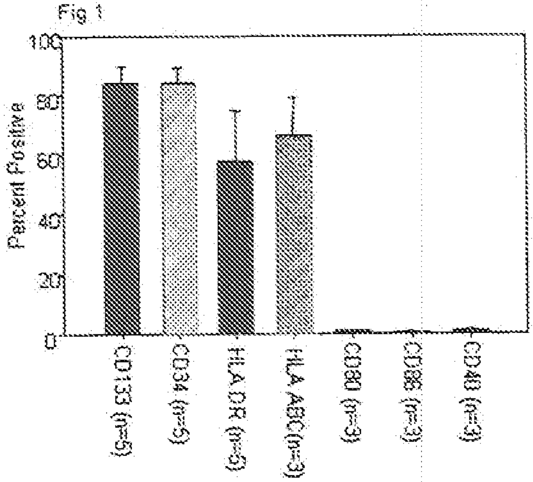
ATTACHEMENT A

Fig 1



UCB CD133+ cells express HLA class I and II antigens and do not express co-stimulatory antigens (Fig 1). UCB CD133$^+$ HSC co-express CD34 and express class I (HLA-ABC) and class II (HLA-DR) but **lack expression of co-stimulatory antigens** including **CD40, CD80 and CD86**. Co-stimulatory antigens are required for full T-cell activation and their absence on antigen presenting cells (APC) leads to T-cell anergy, due to absent binding and co-stimulation of T-cells via CD28 engagement [9]. Nevertheless, whether the immunogenicity of a CD133$^+$ allogeneic therapeutic cellular product is advantageous to augment angiogenesis by recipient endothelial cells *in situ* or potentially deleterious in dampening angiogenesis response or responsible for worsening vascular ischemia via allogeneic inflammatory responses is unknown; and warrants administration of low cell doses of CD133 therapeutic cellular product in a phase I dose escalation approach.

UCB CD133$^+$ HSC and human MSC synergistically promote vasculogenesis in response to ischemia. Preliminary studies conducted in Dr. Laughlin's laboratory at Case Western Reserve University tested human bone marrow (BM)-derived mesenchymal stem cells (MSC) injected alone and in combination with UCB-derived CD133$^+$ HSC. Significantly enhanced blood flow and histologic evidence of angiogenesis was noted in this NOD.SCID *in vivo* hindlimb femoral ligation model (Fig 2). Femoral ligation and resection was performed and study animals were randomized to one of three treatment groups. Group 1, control, was treated with injection of media (0.02 ml). Group 2 animals received third passage human MSC ($1x10^6$ in 0.02 ml). Group 3 animals received both UCB CD133$^+$ HSC and MSC at an equivalent total cell dose ($0.5x10^6$ + $0.5x10^6$ in 0.02 ml; total combined human cell dose $1x10^6$ in 0.04 ml). The animals were survived for 6 weeks. There were significant differences in the Doppler blood flow ratio measured at day 42 among the three conditions. Pair-wise comparison revealed significantly higher blood flow measured in animals injected with both MSC and UCB 133$^+$ HSC compared with those animals treated with MSC alone ($p<0.05$). Taken together, these preliminary reports point to potential synergy between endogenous endothelial, inflammatory, and stromal cells and administered hematopoietic stem and mesenchymal cells in mediating murine angiogenesis responses to ischemic vessel injury.

Fig 2